

REVIEW

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Regarding dissertation for awarding the educational and scientific degree "Doctor" of Dr. Lyuben Lyudmilov Stoev, PhD student free training at the Department "General and clinical pathology, forensic medicine and deontology" in the Faculty of Medicine at Medical University of Varna, on topic „ Expression of tetraspanin markers in benign prostatic hyperplasia and prostatic carcinoma" with supervisor prof. Maria Angelova Tzaneva, MD, PhD.

The proposed for review by me of dissertation on topic „ Expression of tetraspanin markers in benign prostatic hyperplasia and prostatic carcinoma" with author Dr. Lyuben Stoev is topical. The benign prostatic hyperplasia and prostatic carcinoma are ones of the most common diseases of urogenital system in male. The benign prostatic hyperplasia and prostatic carcinoma are ones of the most common diseases of urogenital system in men. According to the literature, prostatic cancer is the second most common malignancy in men and one of the most common causes of cancer-related mortality as a result of local tumor progression and metastasis. A number of risk factors, including age, family history, some genetic mutations, hormonal and growth factors, are involved in the oncogenesis of prostatic cancer, but the exact mechanisms remain unclear. Although the clinical and morphological parameters used in routine oncology practice play a key role in determining patients' prognosis and choice of therapy, their ability to predict the risk of tumor progression and metastasis is limited. Modern medicine is constantly striving to improve diagnostic and treatment methods in oncology by studying new molecules that would be important as prognostic factors or as potential target molecules for targeted therapy. In recent years, the role of tetraspanin proteins in relation to the invasive and metastatic potential of malignant tumors with different localization has been the subject of intensive study. Tetraspanin molecules have been found to be transmembrane proteins that act as mediators in cellular migration processes and complex cellular interactions with the extracellular matrix. Literature data on the role of tetraspanin markers CD9 and CD151 as suppressors or promoters of tumor

progression in different neoplasms are contradictory. Studying the expression of these markers in prostatic cancer would contribute to a more accurate prediction of its biological behavior and the conduction of individualized therapy in order to improve the prognosis of many patients with this cancer.

In this direction is the dissertation work of Dr. Lyuben Stoev. The presented dissertation meets the requirements for structure and volume of the Faculty of Medicine, MU-Varna. It is written on 115 pages, designed in the following sections: Literature review (34 pages), Aim and purposes (1 page), Material and methods (6 pages), Results (38 pages), Discussion (12 pages), Conclusions (2 pages), Contributions (2 pages), Publications related to the dissertation (1 page) and References (18 pages).

The literature review is devoted to the prostatic carcinoma and synthesizes the state of scientific knowledge at the moment. Dr. Stoev focuses in detail on the epidemiology and risk factors for the development of prostate cancer, the morphological diagnosis of conventional adenocarcinoma and its histological variants; the modern criteria for determining the degree of differentiation and staging systems of prostatic carcinoma, as well as on serum and morphological parameters with prognostic and predictive value. A large part of the review is devoted to the accumulated data in the literature related to the role of tetraspanin proteins and in particular tetraspanins CD 9 and CD 151 in carcinogenesis and tumor metastasis in malignant tumors of different localization, including prostate cancer. The bibliography contains 194 literature sources, of which only 4 - in Bulgarian. The literature citations are up-to-date as over 50% of them are from the last 10 years.

The aim of the dissertation is clearly formulated, namely to analyse the clinico-morphological parameters in patients with non-advanced and advanced prostatic carcinoma in relation with the immunohistochemical expression of tetraspanins CD9 and CD151 and elucidating their role in tumor progression. There are 4 purposes that fully meet the aim. To perform the set purposes, in the study are included 91 patients with prostatic carcinoma and 10 patients with benign prostatic hyperplasia (BPH), histologically diagnosed at the Department "General and clinical pathology, forensic medicine and deontology", MU-Varna. Patients with prostate cancer were divided into two groups - with non-advanced cancer ($n = 50$, without distant metastases / M0) and with advanced cancer ($n = 41$, with evidence of distant metastases / M1 at

diagnosis). In all patients with prostate cancer, histological analysis of the tumor was performed, assessing the histological type of the tumor, the degree of differentiation, the presence of perineural invasion, tumor necrosis and / or cribriform histological structure, as well as the T stage. Immunohistochemical expression of tetraspanins CD9 and CD151 in all included patients (with prostatic carcinoma and BPH) was assessed using the H-score (histological score). The methods used for statistical analysis, as well as the morphological methods of research are adequate to the set aim and purposes, and all the necessary conditions for their implementation were observed.

It was obtained original results, illustrated with 38 figures and 48 tables. An exhaustive analysis of the individual clinical and morphological characteristics of prostatic carcinoma was performed in the both groups of patients (with non-advanced and advanced prostate cancer). It has been found that non-advanced and also advanced prostate cancer is most common in men in the age groups 60-69 and 70-79, and in patients with advanced prostate cancer there is a tendency to increase the Gleason score with age. The results show that the mean PSA value in patients with prostate cancer and distant metastases is significantly higher than in patients with non-advanced cancer. In patients with prostatic carcinoma without distant metastasis a relationship was found between PSA values on the one hand and individual histological parameters, including a cribriform pattern of tumor growth, Gleason score, and perineural invasion. Due to the higher PSA values among the patients with advanced prostate cancer, no such association was found. The incidence of the cribriform growth pattern in the group of patients with advanced prostate cancer was significantly higher compared to that in patients with non-advanced tumor (73% and 54%, respectively). A comparative analysis between Gleason scores on the one hand and M stage (M0 / M1) in patients with prostatic carcinoma also found a relationship between the two variables. In patients with advanced prostate cancer, the proportion of tumors with Gleason score 9 or 10 was 51%, while in the group of patients with non-advanced cancer - 14%. In both groups of patients with prostate cancer (stage M0 and M1), the incidence of histologically established perineural invasion was high - 70% and 68%, respectively. In my opinion, the reported results for the T-stage are inadequately convincing because the histological diagnosis in some of the patients with non-advanced prostate cancer

(18/50) and in all patients with advanced prostate cancer was made on needle biopsy material. The results of the performed immunohistochemical studies show significant differences in the expression of the tetraspanin markers CD 9 and CD151 when comparing the groups with non-advanced and advanced prostate cancer and BPH. The mean cytoplasmic expression of CD 9 in BPH was found to be higher than in non-advanced and advanced prostate cancer, but without a statistically significant difference. Accumulated literature data show that decreased CD9 expression is associated with a worse prognosis in a number of carcinomas of different localization (lobular breast cancer, melanoma, lung and colorectal cancer, neoplasms of the pancreas, ovary and prostate). In the present study, it was reported that in both non-advanced and advanced prostatic tumors, CD9 expression did not show a relationship to the degree of tumor differentiation determined by the Gleason score and the cribriform tumor growth pattern. These results support the thesis that the transpanin marker CD9 has little effect on tumor progression in prostatic carcinoma. The results of the immunohistochemical study of CD 151 expression showed that the highest expression of CD151 was found in prostatic carcinomas with distant metastases, followed by that of prostatic carcinomas with no distant metastases and the lowest in BPH, but the difference was statistically insignificant. The comparative analysis performed in non-advanced prostate cancers showed a positive relationship between CD151 expression levels on the one hand and the cribriform growth pattern, the degree of tumor differentiation assessed by Gleason score and perineural invasion. In advanced prostate carcinomas, there was no statistically significant relationship between CD151 expression in tumor tissue and individual morphological parameters. Based on the obtained results, it is concluded that the transpanin marker CD151 probably participates in tumor growth and metastasis, but not alone, but as a component of tetraspanin-integrin complexes in interaction with other molecules. It should be noted that all the results reported in this dissertation are very well interpreted and compared with those of other authors.

The 11 conclusions made by Dr. Stoev fully correspond to the obtained results. The indicated contributions of the dissertation work are of original or scientific-applied nature and are of direct importance for the modern morphological diagnosis of prostate cancer in everyday clinical practice.

The dissertation abstract of Dr. Stoev meets the requirements according to the Rules for the conditions and order for obtaining scientific degrees of MU-Varna and reflects the main elements of the research and the results in the dissertation.

In connection with the dissertation, the PhD student Dr. Stoev has 2 publications (the both in the scientific journal "Varna Medical Forum") as well as 1 scientific report at a national scientific forum. Dr. Stoev is the first author of the both publications, which is proof of its leading role in the research.

In summary, the dissertation of Dr. Lyuben Lyudmilov Stoev is fully completed, with clear aim and tasks, original results and conclusions about the clinico-morphological characteristics and immunohistochemical expression of tetraspanin markers CD 9 and CD 151 in patients with non-advanced and advanced prostatic carcinoma. It meets the requirements of the Law for the development of the academic staff in our country, the Rules for its implementation and the terms and conditions for obtaining scientific degrees of Medical University of Varna. Therefore, I recommend to the honoured members of the Scientific Jury for conducting the defense to give a positive vote of Dr. Lyuben Lyudmilov Stoev for the award of the educational and scientific degree "Doctor" in the scientific specialty "Pathologoanatomy and Cytopathology".

Reviewer:

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(Prof. Radina Ivanova-Boyanova, MD, PhD, DSci)